Lab 5 Myoglobin Protein Isolation

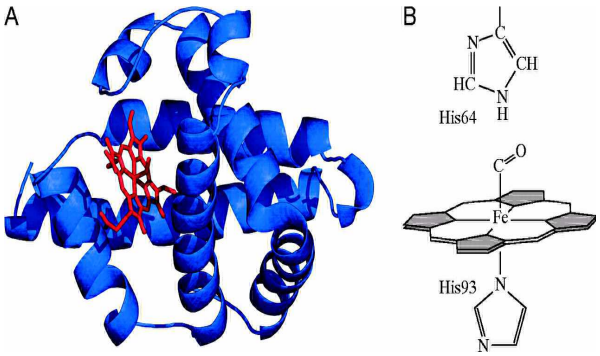
Background

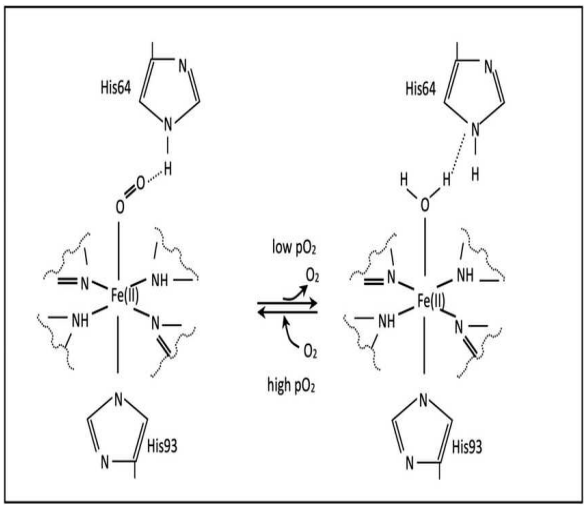
The heme protein myoglobin is found in most muscle tissue. Like hemoglobin (Hb), myoglobin (Mb) contains a heme-bound Fe(II) (Fe^{2+}-O_2-Mb) cation (Oxy-Mb) that can be oxidized to the Fe(III) (Fe^{3+}-H_2O-Mb) form (Met-Mb). The single heme group (iron protoporphyrin IX) is ligated to the protein by histidine residues. For many decades it was believed that the main function of Mb-heme-With a Fe(II) cofactor was to bind O_2, as well as Co, N_2, nitrite, and azide ligands. Recent evidence suggests that the major biological function of Mb may be to catalyze the nitrite/nitric oxide interconversion in muscle.

In vivo, Oxy-Mb and deoxy-Mb (the unligated ferrous state) are the two most common forms. However, in a nonliving system, Oxy-Mb is slowly converted to Met-Mb as the heme-bound O_2 molecules is released and an inactive-site H_2O molecule is bound.

Unlike Hb, which is tetrameric and binds oxygen cooperatively with a Hill coefficient of *n* - 28, Mb is easily extricated from muscle tissue and spectrophotometric characterization of the Fe-heme is facile, as it absorbs strongly in the visible and UV ranges. In fact, the visible absorption of heme-coordinated Fe(II) in Mb is responsible for the reddish appearance of fresh (reduced) meat, whereas the visible absorption of heme-coordinated Fe(III) in metMb is consistent with the brownish color old (oxidized) meat.

It is possible to use chemical agents to duplicate the oxidizing and reducing reactions of living systems. The functionally important state, Oxy-Mb, can be converted to inactive Met-Mb by oxidation in a reaction where an electron from the (ferrous) iron is donated to





the oxidizing agent. Alternatively, Met-Mb can be converted to Oxy-Mb by a reduction reaction in which the heme iron gains an electron from the reducing agent.

The redox reagents can be separated from the Mb sample by gel filtration chromatography to yield homogeneous samples of the muddy brown Met-Mb or bright red Oxy-Mb for detailed spectral analysis (scanning the full range from 700 to 300 nm.

Experiment 1 - Isolation of Mb

Weigh out 10g of burger
Place burger in a centrifuge tube with 20 ml buffer
Mix with a glass rod for 1 min.
Breaks open the cells and release the Mb.
Extensive mixing will release fats & NAs (do not want).
Centrifuge for 15 min at 12,000 x *g*.
Be sure to balance tubes!

After centrifugation should observe a whitish-gray pellet and a reddish supernatant containing the Mb.

Remove supernatant with a Pasteur pipet (not the top fat layer) & transfer to a new centrifuge tube and label.

Obtain to three new tubes.

1. Extract - 1 ml sup. + 3.0 ml of buffer
2. Reduced - transfer ≅ ½ of sup. add ≅ 15 crystals of potassium ferricyanide ($K_6[Fe(CN)_6]$
   Converts Fe^{2+} (ferrous) heme proteins to the Fe^{3+} (ferric) form.
   = Met-Mb
3. Oxidized - transfer the rest of the ≅ ½ sup. add ≅ 20 crystals of sodium dithionite ($Na_2S_2O_4$)
   Reducing agent that will convert Fe^{3+} heme protein to the Fe^{2+} form.
   Should be a bright red Oxy-Mb.

Incubate at RT for 5 min.

Blank NanoDrop with 1µl of buffer. 700-300 nm

Measure the absorbance of your reduced and oxidized Mb samples.
Wipe off the NanoDrop with a KimWipe
Place 1µl of your sample on the NanoDrop and measure

Wipe off the NanoDrop with a KimWipe before transferring your other Mb sample for measurement.

Buffer = 20 mM potassium phosphate